

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Error
1	BRS	L1	5663	fluorescent adj protein	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:39		0	
2	BRS	L3	90	1 same tandem	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:40		0	
3	BRS	L4	26	tandem adj fluorescent adj protein	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:50		0	
4	BRS	L5	37854	monomer or homodimer	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:50		0	
5	BRS	L6	7	3 same 5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:53		0	
6	BRS	L7	2	4 same 5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:54		0	
7	BRS	L8	5672	gfp or rfp or cfp or yfp	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:55		0	
8	BRS	L9	76	8 same tandem	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:56		0	
9	BRS	L10	7	9 same 5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:56		0	
10	BRS	L11	28561	fusion adj protein	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:56		0	
11	BRS	L12	3	11 same (6 or 10)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:58		0	
12	BRS	L13	41622	his adh tag	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 18:59		0	

Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Error
13	BRS	L14 23233 1	enzyme or (g adj protein) or (growth factor) or receptor) or (transcription adj factor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:00			0
14	BRS	L15 17	(13 or 14) same (4 or 3) same 11	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:05			0
15	BRS	L16 110	tsien adj roger.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:06			0
16	BRS	L17 314	campbell adj robert.in.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:06			0
17	BRS	L18 14	(17 or 16) and 4	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:07			0
18	BRS	L19 7	18 and (monomer or homodimer)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/05/2 4 19:07			0

=> d his

(FILE 'HOME' ENTERED AT 19:12:37 ON 24 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA'
ENTERED AT

19:13:00 ON 24 MAY 2003

L1 46908 S FLUORESCENT PROTEIN
L2 2 S TANDEM FLUORESCENT PROTEIN
L3 1 DUPLICATE REMOVE L2 (1 DUPLICATE REMOVED)
L4 36097 S GFP OR RFP OR CFP OR YFP
L5 20109 S L4 (P) L1
L6 197 S L5 (P) TANDEM
L7 5 S L6 (P) (MONOMER OR HOMODIMER)
L8 1 DUPLICATE REMOVE L7 (4 DUPLICATES REMOVED)
L9 1 S L8 NOT L3
L10 144700 S FUSION PROTEIN
L11 35 S L10 (P) L6
L12 9 DUPLICATE REMOVE L11 (26 DUPLICATES REMOVED)
L13 0 S L12 (P) (MONOMER OR HOMODIMER)

FILE 'MEDLINE' ENTERED AT 19:13:00 ON 24 MAY 2003

FILE 'CAPLUS' ENTERED AT 19:13:00 ON 24 MAY 2003
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FILE 'EMBASE' ENTERED AT 19:13:00 ON 24 MAY 2003
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FILE 'SCISEARCH' ENTERED AT 19:13:00 ON 24 MAY 2003
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FILE 'AGRICOLA' ENTERED AT 19:13:00 ON 24 MAY 2003

=> s fluorescent protein
L1 46908 FLUORESCENT PROTEIN

=> s tandem fluorescent protein
L2 2 TANDEM FLUORESCENT PROTEIN

=> duplicate remove l2
DUPLICATE PREFERENCE IS 'CAPLUS, BIOSIS'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L2
L3 1 DUPLICATE REMOVE L2 (1 DUPLICATE REMOVED)

=> d l3 1 ibib abs

L3 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 1999:718875 CAPLUS
DOCUMENT NUMBER: 131:348774
TITLE: ***Tandem*** ***fluorescent*** ***protein***
constructs and their preparation for enzyme assays
INVENTOR(S): Tsien, Roger Y.; Heim, Roger; Cubitt, Andrew
PATENT ASSIGNEE(S): The Regents of the University of California, USA;
Aurora Biosciences Corporation
SOURCE: U.S., 33 pp., Cont.-in-part of U.S. Ser. No. 594,575.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5981200	A	19991109	US 1997-792553	19970131
ES 2177939	T3	20021216	ES 1997-905667	19970131
US 2002164674	A1	20021107	US 2002-57505	20020125

PRIORITY APPLN. INFO.: US 1996-594575 A2 19960131
US 1997-792553 A1 19970131
US 1999-396003 A1 19990913

AB This invention provides ***tandem*** ***fluorescent***
protein construct including a donor fluorescent protein moiety, an
acceptor fluorescent protein moiety and a linker moiety that couples the
donor and acceptor moieties. The donor and acceptor moieties exhibit
fluorescence resonance energy transfer which is eliminated upon cleavage.
The constructs are useful in enzymic assays. Mutant green fluorescent
proteins (GFPs) were created by mutagenesis of the Aequorea victoria GFP.
Polyhistidine tagged tandem green and blue fluorescent proteins were
recombinantly constructed having an inserted peptide sequence including
cleavage recognition sites for many proteases. Cleavage expts. were done
with trypsin, enterokinase and calpain.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 19:17 ON 24 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
19:13:00 ON 24 MAY 2003

L1 46908 S FLUORESCENT PROTEIN
L2 2 S TANDEM FLUORESCENT PROTEIN
L3 1 DUPLICATE REMOVE L2 (1 DUPLICATE REMOVED)

=> s gfp or rfp or cfp or yfp

L4 36097 GFP OR RFP OR CFP OR YFP

=> s l4 (p) l1

L5 20109 L4 (P) L1

=> s l5 (p) tandem

L6 197 L5 (P) TANDEM

=> s l6 (p) (monomer or homodimer)

L7 5 L6 (P) (MONOMER OR HOMODIMER)

=> duplicate remove l7

DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH'

KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n

PROCESSING COMPLETED FOR L7

L8 1 DUPLICATE REMOVE L7 (4 DUPLICATES REMOVED)

=> s l8 not l3

L9 1 L8 NOT L3

=> d l9 1 ibib abs

L9 ANSWER 1 OF 1 MEDLINE
ACCESSION NUMBER: 2002317863 MEDLINE
DOCUMENT NUMBER: 22056088 PubMed ID: 12060735
TITLE: A monomeric red fluorescent protein.
AUTHOR: Campbell Robert E; Tour Oded; Palmer Amy E; Steinbach Paul
A; Baird Geoffrey S; Zacharias David A; Tsien Roger Y
CORPORATE SOURCE: Department of Pharmacology. University of California at San
Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.
CONTRACT NUMBER: 2P30 CA 23100-18 (NCI)
GM 62114 (NIGMS)
NS 27177 (NINDS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE
UNITED STATES OF AMERICA, (2002 Jun 11) 99 (12) 7877-82.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF506025; GENBANK-AF506026; GENBANK-AF506027
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020613
Last Updated on STN: 20020803
Entered Medline: 20020802

AB All coelenterate ***fluorescent*** ***proteins*** cloned to date
display some form of quaternary structure, including the weak tendency of
Aequorea green ***fluorescent*** ***protein*** (***GFP***) to
dimerize, the obligate dimerization of Renilla ***GFP***, and the
obligate tetramerization of the red ***fluorescent*** ***protein***
from Discosoma (DsRed). Although the weak dimerization of Aequorea
GFP has not impeded its acceptance as an indispensable tool of
cell biology, the obligate tetramerization of DsRed has greatly hindered
its use as a genetically encoded fusion tag. We present here the stepwise
evolution of DsRed to a dimer and then either to a genetic fusion of two
copies of the protein, i.e., a ***tandem*** dimer, or to a true
monomer designated mRFP1 (monomeric red ***fluorescent***
protein). Each subunit interface was disrupted by insertion of
arginines, which initially crippled the resulting protein, but red
fluorescence could be rescued by random and directed mutagenesis totaling
17 substitutions in the dimer and 33 in mRFP1. Fusions of the gap
junction protein connexin43 to mRFP1 formed fully functional junctions,

whereas analogous fusions to the tetramer and dimer failed. Although mRFP1 has somewhat lower extinction coefficient, quantum yield and photostability than DsRed, mRFP1 matures >10 times faster, so that it shows similar brightness in living cells. In addition, the excitation and emission peaks of mRFP1, 584 and 607 nm, are approximately 25 nm red-shifted from DsRed, which should confer greater tissue penetration and spectral separation from autofluorescence and other ***fluorescent*** proteins***.

=> d his

(FILE 'HOME' ENTERED AT 19:12:37 ON 24 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 19:13:00 ON 24 MAY 2003

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L1 46908 S FLUORESCENT PROTEIN
L2 2 S TANDEM FLUORESCENT PROTEIN
L3 1 DUPLICATE REMOVE L2 (1 DUPLICATE REMOVED)
L4 36097 S GFP OR RFP OR CFP OR YFP
L5 20109 S L4 (P) L1
L6 197 S L5 (P) TANDEM
L7 5 S L6 (P) (MONOMER OR HOMODIMER)
L8 1 DUPLICATE REMOVE L7 (4 DUPLICATES REMOVED)
L9 1 S L8 NOT L3
```

=> s fusion protein

```
L10 144700 FUSION PROTEIN
```

=> s l10 (p) l6

```
L11 35 L10 (P) L6
```

=> duplicate remove l11

DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L11

```
L12 9 DUPLICATE REMOVE L11 (26 DUPLICATES REMOVED)
```

=> s l12 (p) (monomer or homodimer)

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PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L81 (P) '
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L85 (P) '
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L89 (P) '
L13 0 L12 (P) (MONOMER OR HOMODIMER)
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=> d l12 1-9 ibib abs

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L12 ANSWER 1 OF 9 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002199316 MEDLINE
DOCUMENT NUMBER: 21929620 PubMed ID: 11933014
TITLE: Simultaneous detection of bacteria expressing GFP and DsRed
genes with a flow cytometer.
AUTHOR: Maksimow Mikael; Hakkila Kaisa; Karp Matti; Virta Marko
CORPORATE SOURCE: Department of Biotechnology, University of Turku, Turku,
Finland.
SOURCE: CYTOMETRY, (2002 Apr 1) 47 (4) 243-7.
Journal code: 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020405
Last Updated on STN: 20020801
Entered Medline: 20020731
AB BACKGROUND: In this study, Escherichia coli cells producing red
***fluorescent*** ***protein*** of Discosoma sp. (drFP583 DsRed)
were investigated with flow cytometry by using 488 nm excitation. We also
studied whether green ***fluorescent*** ***protein*** ( ***GFP***
) and drFP583 could be detected simultaneously from a single bacterial
```

cell. METHODS: Plasmids pDsRed and pEGFP were used for the production of drFP583 and enhanced ***GFP***, respectively, in E. coli 061 cells. To produce enhanced ***GFP*** and drFP583 simultaneously, plasmids pG9R and pG19R were constructed. These encode ***tandem*** fusions of enhanced ***GFP*** and drFP583 to ensure similar production levels for both proteins. RESULTS: Bacteria producing enhanced ***GFP*** and drFP583 were found to be brightly green and red fluorescent, respectively. Production of enhanced ***GFP*** and drFP583 ***fusion*** proteins resulted in bacteria that emitted both green and red fluorescence, which was detected easily by a flow cytometer using single laser excitation. Previously reported tetramerization of drFP583 did not restrict its use as a reporter gene, although it matured significantly slower than enhanced ***GFP***. CONCLUSIONS: The results show that enhanced ***GFP*** and drFP583 proteins can be detected simultaneously from single bacteria with a standard flow cytometer with simple optical configuration.

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L12 ANSWER 2 OF 9 MEDLINE DUPLICATE 2
 ACCESSION NUMBER: 2001157621 MEDLINE
 DOCUMENT NUMBER: 21092629 PubMed ID: 11162541
 TITLE: A negative regulator of telomere-length protein trf1 is associated with interstitial (TTAGGG)n blocks in immortal Chinese hamster ovary cells.
 AUTHOR: Krutilina R I; Oei S; Buchlow G; Yau P M; Zalensky A O; Zalenskaya I A; Bradbury E M; Tomilin N V
 CORPORATE SOURCE: Institute of Cytology, Russian Academy of Sciences, Tikchoretskii Av. 4, St. Petersburg, 194064, Russian Federation.
 SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2001 Jan 19) 280 (2) 471-5.
 Journal code: 0372516. ISSN: 0006-291X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200103
 ENTRY DATE: Entered STN: 20010404
 Last Updated on STN: 20021218
 Entered Medline: 20010322

AB Telomeres of mammalian chromosomes are composed of long ****tandem*** repeats (TTAGGG)n which bind in a sequence-specific manner two proteins-TRF1 and TRF2. In human somatic cells both proteins are mostly associated with telomeres and TRF1 overexpression resulting in telomere shortening. However, chromosomes of some mammalian species, e.g., Chinese hamster, have large interstitial blocks of (TTAGGG)n sequence (IBTs) and the blocks are involved in radiation-induced chromosome instability. In normal somatic cells of these species chromosomes are stable, indicating that the IBTs are protected from unequal homologous recombination. In this study we expressed V5-epitope or green ***fluorescent*** protein (***GFP***)-tagged human TRF1 in different lines of mammalian cells and analyzed distribution of the ***fusion*** proteins in interphase nucleus. As expected, transient transfection of human (A549) or African green monkey cells with ***GFP***-N-TRF1 or TRF1-C-V5 plasmids resulted in the appearance in interphase nuclei of multiple faint nuclear dots containing ***GFP*** or V5 epitope which we believe to represent telomeres. Transfection of immortalized Chinese hamster ovary (CHO) cell line K1 which have extremely short telomeres with ***GFP***-N-TRF1 plasmid leads to the appearance in interphase nuclei of large ***GFP*** bodies corresponding in number to the number of IBTs in these cells. Simultaneous visualization of ***GFP*** and IBTs in interphase nuclei of transfected CHO-K1 cells showed colocalization of both signals indicating that expressed TRF1 actually associates with IBTs. These results suggest that TRF1 may serve as general sensor of (TTAGGG)n repeats controlling not only telomeres but also interstitial (TTAGGG)n sequences.

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L12 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
 ACCESSION NUMBER: 2001:219270 BIOSIS
 DOCUMENT NUMBER: PREV200100219270
 TITLE: Characterization of a novel phosphatidylinositol

3-phosphate-binding protein containing two FYVE fingers in tandem that is targeted to the Golgi.

AUTHOR(S): Cheung, Peter C. F. (1); Trinkle-Mulcahy, Laura, Cohen, Philip; Lucocq, John M.

CORPORATE SOURCE: (1) MRC Protein Phosphorylation Unit, University of Dundee, Dow Street, MSI/WTB Complex, Dundee, DD1 5EH: pcheung1@biochem.dundee.ac.uk UK

SOURCE: Biochemical Journal, (1 April, 2001) Vol. 355, No. 1, pp. 113-121. print. ISSN: 0264-6021.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB We have identified a novel protein of predicted molecular mass 40 kDa that contains two FYVE domains in tandem and has therefore been named TAF1 (Tandem FYVE Fingers-1). The protein is expressed predominantly in heart and binds to PtdIns3P specifically, even though the FYVE domains in TAF1 lacks the first Arg of the consensus sequence R(K/R)HHCR, critical for the PtdIns3P binding of other FYVE domains identified so far. The first Arg is replaced by a Thr and Ser in the N-terminal and C-terminal FYVE domains of TAF1 respectively. Mutational analysis indicates that both FYVE domains are required for high affinity binding to PtdIns3P. Cell localization studies using a green fluorescent protein fusion show that TAF1 is localized to the Golgi, and that the Golgi targeting sequence is located within the N-terminal 187 residues and not in either FYVE domain.

L12 ANSWER 4 OF 9 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 2001492811 MEDLINE

DOCUMENT NUMBER: 21426478 PubMed ID: 11536132

TITLE: Enhancement of organophosphorus hydrolase yield in Escherichia coli using multiple gene fusions.

AUTHOR: Wu C F; Valdes J J; Rao G; Bentley W E

CORPORATE SOURCE: Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, MD 20742, USA.

SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (2001 Oct 5) 75 (1) 100-3. Journal code: 7502021. ISSN: 0006-3592.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 20010906 Last Updated on STN: 20020122 Entered Medline: 20011204

AB It was previously shown that organophosphorus hydrolase (OPH) expression and purification could be tracked by fluorescence of green ***fluorescent*** ***protein*** (***GFP***) when synthesized as an N-terminal fusion with ***GFP*** (Cha et al., 2000; Wu et al., 2000). In order to enhance OPH productivity while utilizing the advantage of the reporter protein (***GFP***), two copies of OPH were cloned in ***tandem*** following the ***gfp*** (uv) gene (e.g., ***GFP*** -OPH(n=2)). Both anti- ***GFP*** and anti-OPH Western blots demonstrated that a higher yield was achieved in comparison to the one copy fusion (***GFP*** -OPH). Importantly, the ***fusion*** ***protein*** was still fluorescent as determined via microscopy. In contrast, a fusion containing two copies of OPH without ***GFP***, and an operon fusion of two OPHs with two independent ribosomal binding sites, did not result in a higher yield than one OPH expressed alone. Copyright 2001 John Wiley & Sons, Inc. BioTechnol Bioeng 75: 100-103, 2001.

L12 ANSWER 5 OF 9 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 2001335569 MEDLINE

DOCUMENT NUMBER: 21296123 PubMed ID: 11403491

TITLE: Comparative studies on discrete and concatemeric DNA-sepharose columns for purification of transcription factors.

AUTHOR: Gadgil H; Taylor W L; Jarrett H W

CORPORATE SOURCE: Department of Biochemistry, University of Tennessee, Memphis 38163, USA.

CONTRACT NUMBER: GM43609 (NIGMS)
 SOURCE: JOURNAL OF CHROMATOGRAPHY. A, (2001 May 11) 917:1-2) 43-53.
 Journal code: 9318488.
 PUB. COUNTRY: Netherlands
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200110
 ENTRY DATE: Entered STN: 20011029
 Last Updated on STN: 20011029
 Entered Medline: 20011025

AB Concatemers, ***tandem*** copies of DNA elements ligated together, are widely used for the DNA affinity chromatography of transcription factors. Purification of different transcription factors using discrete, concatemeric and T18:A18 tailed DNA affinity columns was studied. Columns having a discrete DNA sequence bound by cytidylic-adenylic-adenylic-thymidylic oligonucleotide (CAAT) enhancer binding protein (C/EBP) yields significantly more green ***fluorescent*** ***protein*** -C/EBP (***GFP*** -C/EBP) ***fusion*** ***protein*** than a concatemeric DNA column made from five ***tandem*** repeats of the same DNA sequence. For lac repressor protein, the concatemeric and T18:A18 tailed columns show greater retention times than a discrete, untailed DNA affinity column. It was observed that the T18:A18 tailed column gives better resolution than either the discrete or concatemeric columns, of mixtures containing both lac repressor and ***GFP*** -C/EBP. Discrete concatemeric and T18:A18 tail columns all bound the Sp1 transcription factor and showed similar retention. The T18:A18 tailed column gives higher yield for Sp1 than the other columns. Our study shows concatemeric columns do not have any distinct advantage for the three different transcription factors we studied including Sp1, the original justification for the concatemeric approach.

L12 ANSWER 6 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI

ACCESSION NUMBER: 2001:409561 SCISEARCH

THE GENUINE ARTICLE: 431CC

TITLE: Comparative studies on discrete and concatemeric DNA-sepharose columns for purification of transcription factors

AUTHOR: Gadgil H; Taylor W L; Jarrett H W (Reprint)

CORPORATE SOURCE: Univ Tennessee, Dept Biochem, 858 Madison Ave, Memphis, TN 38163 USA (Reprint); Univ Tennessee, Dept Biochem, Memphis, TN 38163 USA

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF CHROMATOGRAPHY A, (11 MAY 2001) Vol. 917, No. 1-2, pp. 43-53.
 Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
 ISSN: 0021-9673.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 16

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Concatemers, ***tandem*** copies of DNA elements ligated together, are widely used for the DNA affinity chromatography of transcription factors. Purification of different transcription factors using discrete, concatemeric and T-18:A(18) tailed DNA affinity columns was studied. Columns having a discrete DNA sequence bound by cytidylic-adenylic-adenylic-thymidylic oligonucleotide (CAAT) enhancer binding protein (C/EBP) yields significantly more green ***fluorescent*** ***protein*** -C/EBP (***GFP*** -C/EBP) ***fusion*** ***protein*** than a concatemeric DNA column made from five ***tandem*** repeats of the same DNA sequence. For lac repressor protein, the concatemeric and T-18:A(18) tailed columns show greater retention times than a discrete, untailed DNA affinity column. It was observed that the T-18:A(18) tailed column gives better resolution than either the discrete or concatemeric columns, of mixtures containing both lac repressor and ***GFP*** -C/EBP. Discrete concatemeric and T-18:A(18) tail columns all bound the Sp1 transcription factor and showed similar retention. The T-18:A(18) tailed column gives higher yield for Sp1 than the other columns. Our study shows concatemeric columns do not have any distinct advantage for the three different transcription factors

L12 ANSWER 7 OF 9 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999340064 MEDLINE
DOCUMENT NUMBER: 99340064 PubMed ID: 10409682
TITLE: On the maximum size of proteins to stay and fold in the cavity of GroEL underneath GroES.
AUTHOR: Sakikawa C; Taguchi H; Makino Y; Yoshida M
CORPORATE SOURCE: Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta, Yokohama 226-8503, Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jul 23) 274 (30) 21251-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990910
Last Updated on STN: 19990910
Entered Medline: 19990826
AB GroEL encapsulates non-native protein in a folding cage underneath GroES (cis-cavity). Here we report the maximum size of the non-native protein to stay and fold in the cis-cavity. Using total soluble proteins of Escherichia coli in denatured state as binding substrates and protease resistance as the measure of polypeptide held in the cis-cavity, it was estimated that the cis-cavity can accommodate up to approximately 57-kDa non-native proteins. To know if a protein with nearly the maximum size can complete folding in the cis-cavity, we made a 54-kDa protein in which green ***fluorescent*** ***protein*** (***GFP***) and its blue fluorescent variant were fused ***tandem***. This ***fusion*** ***protein*** was captured in the cis-cavity, and folding occurred there. Fluorescence resonance energy transfer proved that both ***GFP*** and blue ***fluorescent*** ***protein*** moieties of the same fused protein were able to fold into native structures in the cis-cavity. Consistently, simulated packing of crystal structures shows that two native ***GFPs*** just fit in the cis-cavity. A ***fusion*** ***protein*** of three ***GFPs*** (82 kDa) was also attempted, but, as expected, it was not captured in the cis-cavity.

L12 ANSWER 8 OF 9 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2000039773 MEDLINE
DOCUMENT NUMBER: 20039773 PubMed ID: 10574616
TITLE: Subcellular localization of interferon-inducible Myc/stat-interacting protein Nmi is regulated by a novel IFP 35 homologous domain.
AUTHOR: Lee N D; Chen J; Shpall R L; Naumovski L
CORPORATE SOURCE: Department of Pediatrics, Stanford Medical Center, CA 94305, USA.
SOURCE: JOURNAL OF INTERFERON AND CYTOKINE RESEARCH, (1999 Nov) 19 (11) 1245-52.
Journal code: 9507088. ISSN: 1079-9907.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991221
AB Nmi was initially identified through a yeast two-hybrid interaction with N-Myc but it also interacts with c-Myc, Max, Fos, and several other transcription factors, including signal transducer and activator of transcription (Stat) proteins. Nmi is an interferon (IFN)-inducible protein with 25% amino acid identity to the IFN-inducible protein IFP 35. We have found that this homology consists of a novel domain of approximately 90-92 amino acids (aa) that is repeated in ***tandem*** in each protein. This region, termed Nmi/IFP 35 domain (NID), is important for subcellular localization of Nmi. Full-length Nmi protein or deletion constructs containing a single NID are localized to the

cytoplasm, but amino-terminal Nmi fragments of up to 92 aa containing neither NID are nuclear. Fusion of the amino-terminal end of Nmi to pyruvate kinase, an exclusively cytoplasmic protein, results in a cytoplasmic ***fusion*** ***protein***, suggesting that the amino-terminal end of Nmi does not contain a classic nuclear localization signal (NLS). Fusion of the amino-terminal end of Nmi to green ***fluorescent*** ***protein*** (***GFP***), which is normally found in both nuclear and cytoplasmic compartments, does not alter ***GFP*** distribution, whereas fusion of a single NID to ***GFP*** targets the fusion to the cytoplasm. Fusion of a nuclear localization signal (NLS) to full-length Nmi or NID repeats targets the hybrid to the nucleus, suggesting that a strong NLS is dominant to the cytoplasmic localization function of NID. NID may mediate cytoplasmic localization of the full-length Nmi protein through NID-NID protein interactions as demonstrated by yeast two-hybrid assay, immunoprecipitation, and the presence of Nmi in a high molecular weight protein complex. These results suggest that Nmi is composed of a modular structure with an amino-terminal domain that when separated from the rest of the protein is nuclear. The carboxy-terminal two thirds of the protein is composed of two NID that mediate cytoplasmic localization of the full-length protein.

L12 ANSWER 9 OF 9 MEDLINE DUPLICATE 7
 ACCESSION NUMBER: 1998330438 MEDLINE
 DOCUMENT NUMBER: 98330438 PubMed ID: 9664035
 TITLE: YEB3/VAC8 encodes a myristylated armadillo protein of the Saccharomyces cerevisiae vacuolar membrane that functions in vacuole fusion and inheritance.
 AUTHOR: Pan X; Goldfarb D S
 CORPORATE SOURCE: Department of Biology, University of Rochester, Rochester, New York 14627, USA.. dasg@uhura.cc.rochester.edu
 CONTRACT NUMBER: GM40362 (NIGMS)
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 PUB. COUNTRY: ENGLAND: United Kingdom
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 AB Armadillo (Arm) repeat proteins such as beta-catenin and alpha-karyopherin (importin) are thought to mediate the docking of cargo at membrane-associated cytoskeletal elements. YEB3 encodes an uncharacterized Saccharomyces cerevisiae protein that contains eleven ***tandem*** Arm repeats. While YEB3 is nonessential for growth, yeb3delta cells accumulated numerous small vacuoles and are defective in vacuolar inheritance. A functional Yeb3p-green ***fluorescent*** ***protein*** (***GFP***) chimera localized to vacuolar membranes. Confocal microscopy revealed that Yeb3p- ***GFP*** is localized over the surface of the vacuole, but is concentrated approximately 5- to 7-fold in bands located between clustered vacuoles. N-terminal myristylation of Yeb3p is required for vacuolar localization. The first 69 amino acids of Yeb3p were sufficient to target a ***GFP*** reporter protein to the vacuolar membrane; however, this ***fusion*** ***protein*** also localized to the plasma membrane, indicating that additional sequence is required for exclusive steady state vacuolar localization. By analogy to the function of beta-catenin in cell-cell adhesion, alpha-karyopherin in nuclear transport, and smgGDS in the control of ras-like GTPases, Yeb3p may provide a link between vacuoles and the actin cytoskeleton during vacuolar inheritance and fusion and perhaps mediate the assembly of a GTPase regulated docking complex.

=> d his

(FILE 'HOME' ENTERED AT 19:12:37 ON 24 MAY 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 19:13:00 ON 24 MAY 2003

L1 46908 S FLUORESCENT PROTEIN
 L2 2 S TANDEM FLUORESCENT PROTEIN

L3 1 DUPLICATE REMOVE L2 (1 DUPLICATE REMOVED)
 L4 36097 S GFP OR RFP OR C OR YFP
 L5 20109 S L4 (P) L1
 L6 197 S L5 (P) TANDEM
 L7 5 S L6 (P) (MONOMER OR HOMODIMER)
 L8 1 DUPLICATE REMOVE L7 (4 DUPLICATES REMOVED)
 L9 1 S L8 NOT L3
 L10 144700 S FUSION PROTEIN
 L11 35 S L10 (P) L6
 L12 9 DUPLICATE REMOVE L11 (26 DUPLICATES REMOVED)
 L13 0 S L12 (P) (MONOMER OR HOMODIMER)

=> log y

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FULL ESTIMATED COST

45.67

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STN INTERNATIONAL LOGOFF AT 19:18:33 ON 24 MAY 2003